

CA Sub D1  
1. (Twice amended) An isolated protein encoded by a nucleic acid molecule that hybridizes [under stringent conditions] to the complement of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, and SEQ ID NO:33, under conditions  
5 comprising (a) hybridizing in 2X SSPE, 1% Sarkosyl, 5X Denhardts and 0.1 mg/ml denatured salmon sperm and (b) washing in a solution comprising 2X SSPE and 1% Sarkosyl at 55°C; wherein said protein elicits an immune response against a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10,  
10 SEQ ID NO:11, SEQ ID NO:31, and SEQ ID NO:34.

8. (Reiterated) An isolated protein comprising a *D. immitis* astacin metalloendopeptidase protein.

CA Sub D2  
11. (Twice amended) The protein of Claim 1, wherein said protein comprises at least a portion of at least one amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:31, SEQ ID NO:34, wherein said portion elicits an  
5 immune response against a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:31, and SEQ ID NO:34; and wherein said portion comprises an at least 9 contiguous amino acid region of an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID

CA  
Continue  
Sub D2  
cont

10 NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:31, and SEQ ID NO:34.

---

12. (Reiterated) The protein of Claim 1, wherein said protein comprises an extended zinc-binding domain motif.

13. (Reiterated) The protein of Claim 1, wherein said protein is produced by a process comprising culturing in an effective medium a recombinant cell transformed with a nucleic acid molecule encoding said protein to produce said protein.

---

CB  
Sub D3

16. (Twice amended) A therapeutic composition for protecting an animal from disease caused by a parasite, said parasite being susceptible to an inhibitor of an astacin metalloendopeptidase, said therapeutic composition comprising at least one protective compound selected from the group consisting of:

5 (a) an isolated astacin metalloendopeptidase protein encoded by a nucleic acid molecule that hybridizes [under stringent conditions] to the complement of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, and SEQ ID NO:33 under conditions comprising (a) hybridizing in 2X SSPE, 1% Sarkosyl, 5X Denhardts and

10 0.1 mg/ml denatured salmon sperm and (b) washing in a solution comprising 2X SSPE and 1% Sarkosyl at 55°C; and

(b) an isolated protein having an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7,

Sub 13  
bmt  
15  
SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:31, and SEQ ID NO:34, or a homolog thereof, wherein said homolog has an at least about [7]9 contiguous amino acid region [identical in sequence to a 7 contiguous amino acid region] of an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:31, and SEQ ID NO:34; and

(c) an antibody that selectively binds to a protein comprising a *D. immitis* astacin metalloendopeptidase protein].

17. (Reiterated) The composition of Claim 16, wherein said composition further comprises at least one component selected from the group consisting of an excipient, an adjuvant and a carrier.

18. (Reiterated) The composition of Claim 16, wherein said parasite comprises a tissue-migrating helminth.

19. (Reiterated) The composition of Claim 16, wherein said therapeutic composition protects an animal from heartworm infection.

20. (Once Amended) A method to protect an animal from disease caused by a parasite, said parasite being susceptible to an inhibitor of an astacin metalloendopeptidase, said method comprising administering to said animal a therapeutic composition comprising at least one protective compound selected from the group consisting of:

5        (a) an isolated astacin metalloendopeptidase protein encoded by a nucleic acid molecule that hybridizes to the complement of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, and SEQ ID NO:33 under conditions comprising (a) hybridizing in 2X SSPE, 1% Sarkosyl, 5X Denhardts and 0.1 mg/ml denatured salmon sperm and (b) washing in a solution comprising 2X SSPE and 1% Sarkosyl at 55°C; and

10        (b) an isolated protein having an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:31, and SEQ ID NO:34, or a homolog thereof, wherein said homolog has an at least about 9 contiguous amino acid region of an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:31, and SEQ ID NO:34 [an isolated parasite astacin metalloendopeptidase protein; an anti-parasite astacin metalloendopeptidase antibody; and an inhibitor of astacin metalloendopeptidase activity identified by its ability

15

20        to inhibit parasite astacin metalloendopeptidase activity of said protein].

21.        (Reiterated) The method of Claim 20, wherein said parasite comprises a tissue-migrating helminth.

22.        (Reiterated) The method of Claim 20, wherein said disease comprises heartworm infection.

23. (Reiterated) A method to identify a compound capable of inhibiting astacin metalloendopeptidase activity of a parasite, said method comprising:

- 5 (a) contacting an isolated [parasite astacin metalloendopeptidase protein] protein encoded by a nucleic acid molecule that hybridizes to the complement of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, and SEQ ID NO:33, under conditions comprising (a) hybridizing in 2X SSPE, 1% Sarkosyl, 5X Denhardtts and 0.1 mg/ml denatured salmon sperm and (b) washing in a solution comprising 2X SSPE and 1% Sarkosyl at 55°C; with a putative
- 10 inhibitory compound under conditions in which, in the absence of said compound, said astacin metalloendopeptidase protein has astacin metalloendopeptidase activity; and
- (b) determining if said putative inhibitory compound inhibits said activity.

24. (Once Amended) A test kit to identify a compound capable of inhibiting astacin metalloendopeptidase activity of a parasite, said test kit comprising an isolated [parasite astacin metalloendopeptidase protein] protein encoded by a nucleic acid molecule that hybridizes to the complement of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, and SEQ ID NO:33, under conditions comprising (a) hybridizing in 2X SSPE, 1% Sarkosyl, 5X Denhardtts and 0.1 mg/ml denatured salmon sperm and (b) washing in a solution comprising 2X SSPE and 1% Sarkosyl at 55°C; and a means for

05  
Sub.  
E6

CS  
Amended

10

~~determining the extent of inhibition of said activity in the presence of a putative inhibitory~~

~~compound.~~

CS

Sub 24

25. (Once Amended) An isolated protein having an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:31, and SEQ ID NO:34, or a homolog thereof, wherein said homolog has an at least 5 [about] 9 contiguous amino acid region [identical in sequence to a 9 contiguous amino acid region] of an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:31, and SEQ ID NO:34.

Please add new Claim 26, as follows:

- 11 26. (New) The protein of Claim 25, wherein said protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:31, and SEQ ID NO:34.

#### REMARKS

1. Election/Restriction Requirement under 35 U.S.C. § 121

The Examiner considered the Applicants' arguments for traversal of the restriction between Groups I-IV. With respect to the Applicants' arguments regarding the restriction between Group I and Groups III and IV, the Examiner did not find the arguments persuasive and therefore the